

PATHOGENIC VARIABILITY OF FOLIAR NEMATODE,

APHELENCHOIDES BESSEYI CHRISTIE, 1942

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ABSTRACT

Replicated pot culture experiment was conducted in green house benches of Department of Nematology, College of Agriculture, OUAT, Bhubaneswar to study the pathogenic variability of foliar nematode, *Aphelenchoides besseyi*. Population of *A. besseyi* from tuberose and paddy were separately multiplied monoxenically on PDA with *Fusarium moniliforme*. Population of both the paddy and tuberose isolates were cross inoculated along with parental inoculation. The result of the present investigation indicated variation in the symptomatology specific to host plant but not nematode isolates. Greasy leaf, snipping, rotting and floret necrosis were specific to tuberose plant where as rolled leaf tip was specific only to paddy crop. Symptoms like white tip and whip like appearance was common to both paddy and tuberose. The present study indicated the pathogenic variability to the host rather than the isolates of *A. besseyi*. Thus the paddy and tuberose isolates were the same.

KEYWORDS: Pathogenic Variability of Foliar Nematode, *Aphelenchoides besseyi*, *Fusarium moniliforme*

INTRODUCTION

The white tip nematode, *Aphelenchoides besseyi* was first reported by Dastur (1936) from the then Central Provinces of India presently known as Madhya Pradesh, where it had reached an epidemic proportion. Subsequently the same nematode was reported from almost all the rice growing countries of the world. Christie (1942) also reported *A. besseyi* to cause summer dwarf of strawberries in which the symptoms included deformed leaves, short petioles and leaflets, often with unsymmetrical leaves, usually crinkled with margins cupping upwards in young leaves and downwards in older leaves. Petioles, veins and underside of leaflet are often purple to reddish in color. If the main bud was killed, the plants failed to produce more leaves and often died. The same nematode species infecting rice produces different types of symptoms. The apiece of the infected leaves turn white or yellow up to 2–5 cm. These areas gradually turn brown or black and frayed. Such leaves appear whip like in the dry season and curl or shrivel during wet weather season. Flag leaf is often characteristically twisted at the base of the panicle. Infested panicles are short and bear fewer spikelets. Terminal tissues of the spikelets are severely reduced and resulted in the production of deformed grains. The ripening of the panicle is delayed and secondary panicles may arise from the lower nodes if the panicle is sterile. This nematode induces an average yield loss up to 30-50% in rice (Swain, 1987).

REVIEW OF LITERATURE

Pedramfar *et al.*, (2001) found plant parasitic nematodes of the rice fields in Guilan province (Iran), about 75 soil and root samples were collected from different fields in 1995 and 1996. The nematodes were extracted by centrifugal sugar floatation method. Ten known plant parasitic nematodes those belonging to 8 genera were identified including *A. besseyi*, *A. bicaudatus*, *Basiria graminophila*, *Helicotylenchus crenacaudatus*, *H. digitiformis*, *Heterodera oryzae* and *Tylenchorhynchus annulatus*.

Khan and Pal (2001) reported the occurrence and distribution of plant parasitic nematodes in tuberose, *Polianthes tuberosa* (cultivars Single and Double) growing in West Bengal, India. *A. besseyi*, *Meloidogyne incognita* and *Rotylenchulus reniformis* were identified in all growing regions studied. *A. besseyi* which was mostly recovered from flowering stalks and caused the most serious damage in Ranaghat and Haringhata regions

MATERIALS AND METHODS

Experiments on Pathogenic variability of *Aphelenchoides besseyi* were conducted in the net-house and laboratory condition of Department of Nematology, College of Agriculture, OUAT and Bhubaneswar. In order to have a clear view into different components of the experiments, the various materials and methods used in the investigation were described here under.

- **Collection of Inoculums**

The foliar nematode, *A. besseyi* is found to infect graminaceous host plants like paddy and non-graminaceous host plants, tuberose (*Polianthes tuberosa*) in the state of Odisha. Infected paddy seeds of variety 'Swarna' were collected from OUAT Central farm. Similarly, infected leaves of tuberose exhibiting greasy leaf symptoms and spikelets exhibiting floret necrosis were collected from farmers' field.

- **Culturing of Nematodes**

- **Preparation and Sterilization of Media**

Prepared dry potato dextrose agar (20g) obtained from high media was dissolved in a conical flask containing 1liter of distilled water. The flask was plugged in with non-absorbent cotton and sterilized in an autoclave at 121.6 °C with 15lb pressure for (15-20) minutes. After autoclaving, the flask was taken out of autoclave and stored in refrigerator for further use as and when required.

- **Inoculation of Media with Fungus**

About (10-15) ml of melted PDA was poured into sterilized petridishes. Stalk culture of *Fusarium moniliforme* obtained from Department of Plant Pathology was used as the standard inoculums. Loop full of fungal mycelia mat was transferred aseptically from stalk culture to the petridishes containing PDA inside the laminar air flow. Petridishes were incubated for (3-4) days inside incubator at 30 °C, for sufficient growth of fungi. (Plate 1)

- **Inoculation and Multiplication of *A. besseyi***

Two sets of petridishes were used for multiplication of *A. besseyi* grown in *F. moniliforme* culture. One set of petridishes were used to grow isolates of paddy and the other set for tuberose. Infected paddy seeds and spikelets of tuberose were collected and teased upon in petridishes containing water supported by wire gauge for recovery of *A. besseyi*

for overnight. Recovered nematodes from each isolates were passed through several changes of sterile distilled water to make them contamination free. Axenized 10 pre-adult stages of *A. besseyi* were handpicked and transferred into a drop for sterile water on a corner of slide coated with paraffin wax. The drop of water containing axenized nematodes were aseptically transferred to fungal cultures in petridishes. Petridishes were kept inside incubator for 2 weeks for multiplication of *A. besseyi*. Periodically petridishes were taken out of incubator and were added with few drops of sterile water to prevent drying of the media and facilitate the movement and activities of *A. besseyi*. After a period of 2 weeks the petridishes were having large number of *A. besseyi* population (Plate 2) which was further used for cross inoculation studies to rice and tuberose.

- **Isolation of Nematodes from Culture Media**

Nematodes were isolated from cultured petridishes by placing few drops of sterile water inside the petridishes, shaking the petridishes for some time and inverting the petridishes over another container two collects the nematodes in water.

- **Surface Sterilization of Earthen pots**

Forty earthen pots of 10" diameter were surface sterilized by pouring hot water inside the pots and dried in open.

- **Preparation and Sterilization of Pot Mixture**

Field soil from central farm OUAT, Bhubaneswar was collected, clods and plant debris were removed and mixed with compost in a ratio of 2:1. The pot mixture was heaped on a cemented floor and treated with 8% formaldehyde solution, mixed thoroughly and heaped again by covering with transparent polythene. The soil mixture was kept as such for (3-4) days after which polythene was removed and soil was spread in thin layers for about (2-3) days for complete volatilization of traces of formaldehyde solution. Required amounts of N, P, K fertilizers were added to the pot mixture and mixed thoroughly. Sterilized soil mixture was filled up to the brim of 40 earthen pots each containing 3kg of soil.

- **Sowing/ Planting**

The 40 pots filled with sterilized pot mixture were arranged in 4 rows of 10 each. Healthy seeds of paddy variety Swarna were sown in 20 pots containing pot mixture. Healthy tuberose bulbs (var. Calcutta single) of uniform size were planted inside another 20 earthen pots containing soil mixture. Watering of pots was done at regular interval for germination/ sprouting of seeds.

- **Treatment Assignment and Nematization of Plants**

The pots comprising of 40 plants were assigned into four treatments each with 10 replications. About 3000 pre-adult stages of *A. besseyi* from fungal culture of paddy as well as tuberose isolates were inoculated near the leaf sheath of tuberose and paddy

RESULTS

A pot culture study was under taken in the net house of Department of Nematology, College of Agriculture, OUAT, BBSR, during 2013-14 to assess the pathogenic variability of *Aphelenchoides besseyi*. Host isolates of *A. besseyi* were obtained from paddy and tuberose. These host isolates were maintained and cultured monoxenically on *Fusarium moniliforme*. Paddy variety 'Swarna' and tuberose cultivar, 'Calcutta single' were used as the test plant species to study

pathogenic variability through cross inoculation. Observations on the incidence of disease symptoms and recovery of nematodes were made at an interval of 15 days after sowing starting from 15 days after inoculation. Data were arranged in a tabular form according to types of symptoms exhibited on test plant species by different host isolates. Recovery of nematodes thus obtained at different days after sowing were analyzed and presented in tabular form.

Disease Incidence and Nematode Recovery 45 DAS

Result on the incidence of disease symptoms induced by *A. besseyi* is presented (Table-1). White tip symptoms in the form of upper to 2-5 cm apices appeared on paddy leaves inoculated with isolates of paddy and tuberose. However, such symptoms were not observed on tuberose by both the isolates. On the contrary, greasy leaf symptoms were exhibited on tuberose leaves inoculated with both the isolates but not in paddy. Symptoms of rolled leaf tip, whip like appearance, twisting of flag leaf, snipping, rotting of leaves and floret necrosis were not recorded on paddy as well as tuberose.

Table 1: Incidence of Disease Symptoms in Plants by Isolates of *Aphelenchoides besseyi* through Cross Inoculation (45 DAS)

Symptomatology	T1	T2	T3	T4
White tip	+	-	+	-
Rolled leaf tip	-	-	-	-
Whip like appearance	-	-	-	-
Twisting of flag leaf base	-	-	-	-
Greasy leaf	-	+	-	+
Snipping	-	-	-	-
Rotting of leaves	-	-	-	-
Floret necrosis	-	-	-	-

(+) indicates the appearance of a particular symptoms.

(-) indicates the absence of a particular symptoms.

Recovery of nematodes from host isolate (Table-9) indicated variations in their number. Analysis of variance indicated significant differences in the recovery of nematodes of host isolates in paddy as well as tuberose. Highest numbers of nematodes (56) were recorded in tuberose inoculated with paddy isolate followed by tuberose inoculated with tuberose isolate which were at par with each other and significantly difference from each other. Lowest numbers of nematodes were (28) recovered in paddy inoculated with tuberose isolate.

DISCUSSIONS

White tip nematode, *A. besseyi* is an above ground plant parasite infecting large number of cultivated land species including ornamental crops. The first report of this nematode species by Dastur (1936) given a detail account of the disease in the rice and followed by reports of wide spread occurrence of this disease through the world by various workers. This important plant parasitic nematode species caused discoloration of leaf tissue, inhibition of young buds, shoots and primary leaf rot symptoms (Holtzmann, 1968). Subsequently Trujillo in the same year observed water soaked spots near the midrib of leaves, black greasy leaf spot resulting in rotting and snipping of leaves. Chakraborti and Ghosh (1993) have reported floral malady of tuberose from West Bengal by this important nematode species. Swain (1999) also reported this important disease of tuberose by *A. besseyi*. Most of the tuberose cultivation in Odisha and West Bengal is carried out in rice fallows. It was believed that the same nematode species infecting rice also infects tuberose. However, in the year 2007 Cuc and

Pilon reported complication in the cultivation of tuberose in the presence of foliar nematode *Aphelenchoides* sp. and most likely *A. besseyi*. Mohanty (1992) reported pathotypes of *A. besseyi* in three geographical isolates on five differential host of rice. It was not certain whether the pathotypes of *A. besseyi* infecting rice infect tuberose or not. Thus, it was felt imperative to investigate populations of *A. besseyi* from both tuberose and paddy through cross inoculation study to confirm the uncertainties in the cultivation of tuberose. Paddy isolates on paddy induced white tip symptoms starting from 45 DAS till harvest of the paddy crop at 150 DAS in all the stages at 15 days interval. Reports of white tip symptoms by paddy isolate on paddy has been reported by several workers Swain (1987), Swostica (1993), Moletti (1997), Togashi and Hoshino (2001) and Lin *et al.*, (2005). Paddy isolate on tuberose did not exhibit white tip symptoms on tuberose till 75 DAS. However, such symptoms appear at 90 DAS and continued till harvest at 150 days. There are no reports on cross inoculation of paddy isolate to tuberose. The late appearance of white tip symptoms on tuberose might be due to late emergence of new leaves from the central axis. Similarly, tuberose isolate inoculated to paddy induced white tip symptoms starting from 45 days onward till 150 days at all intervals. This is in confirmative with the paddy isolate on paddy. Tuberose isolate on tuberose induced white tip symptom at 90 DAS and continued 150 days at 15 days interval. This late appearance of white tip symptoms on tuberose by the parental isolate is also due to late emergence of young leaves from the central axis. White tip symptoms in tuberose as observed in the present study is in corroboration with the findings by Das *et al.*, (2011) and Khan *et al.*, (2012).

SUMMARY AND CONCLUSIONS

Net house and laboratory experiments were conducted to study pathogenic variability of *A. besseyi* infecting tuberose and paddy. *A. besseyi* from paddy as well as tuberose was isolated separately and cultured monoxenically on *Fusarium moniliforme* grown on PDA. Tuberose cultivar 'Calcutta single' and paddy variety 'Swarna' were chosen for cross inoculation studies by both the isolates. Observation on development of symptoms like white tip, rolled leaf tip, whip like appearance, twisting of flag leaf base, greasy leaf, snipping, rotting of leaves and floret necrosis were taken at 15 days interval starting from 45 DAS.

White tip symptoms were common in all treatments irrespective of the isolate as well as the crops. Rolled leaf tip symptoms appeared only in paddy inoculated with both the isolates, indicating two distinct types of reaction whip like appearance was manifested in paddy as well as tuberose irrespective of isolate. Greasy leaf, snipping, rotting of leaves and floret necrosis appeared only in tuberose irrespective of isolates.

Recovery of nematodes at different days after sowing exhibited significant differences. Similarly, recovery in different treatments irrespective of days after sowing including the interaction exhibited significant differences. The result of the present study indicated recovery of nematodes at each days of observation including each treatment. The result of present investigation indicated two distinct types of symptoms on tuberose as well as paddy. Although the symptoms produced on both the test plant species were different from each other, *A. besseyi* from both tuberose and paddy isolates exhibited similar symptoms like white tip and whip like appearance on paddy and tuberose. The present study indicates both the isolates to be the same without any pathogenic variant on paddy and tuberose. However, the differential symptoms produced on two different test plant species is primarily due to composition of the host tissue favoring the invasion and production of host specific symptoms. Basically paddy leaves are thin with more of silica content as compare to tuberose leaf which is fleshy, thick, waxy and more suitable for feeding by *A. besseyi*. Hence, on the basis of the present study the controversy regarding the isolates of tuberose and paddy is solved and the variation in symptoms is attributed to the host.

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APPENDICES

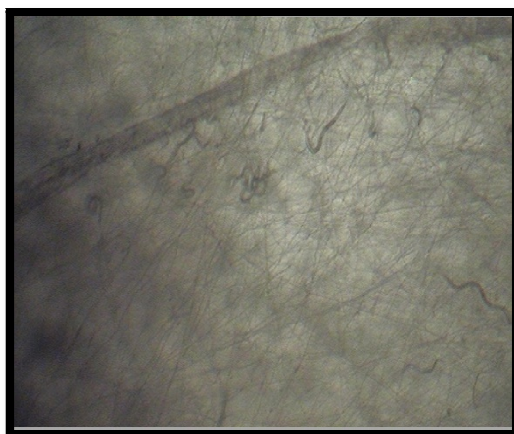


Plate 1: Photograph Showing Growth of *Fusarium moniliforme* on PDA



Plate 2: Photograph Showing *A. besseyi* in Fungal Culture

